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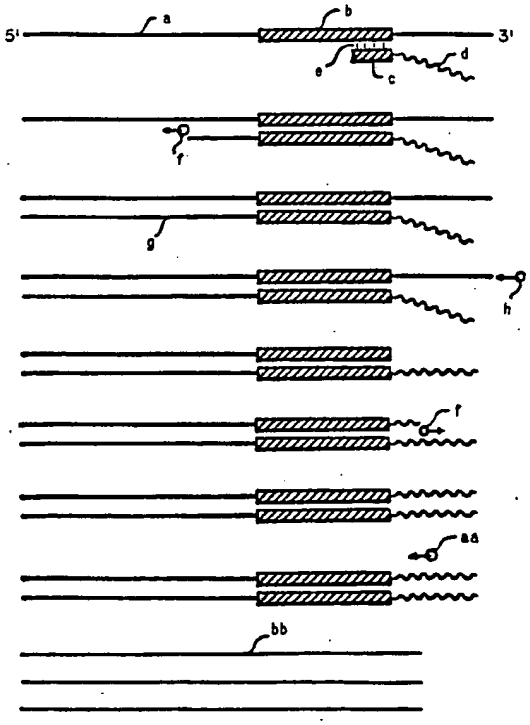
(54) Title: AMPLIFICATION AND DETECTION OF NUCLEIC ACID SEQUENCES

(57) Abstract

The present invention is directed to improved methods for assaying specific nucleic acid sequences in a test sample and the reagents for carrying out the methods. In the general, the methods of the invention involve the synthesis of a double-stranded nucleic acid containing the nucleic acid sequence to be detected and a promoter, the synthesis of a multiplicity of RNA transcripts under the control of the promoter, and the detection of the specific RNA transcripts produced.



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AMENDED CLAIMS

[received by the International Bureau on 28 July 1989 (28.07.89); original claims 1-30 and 36 cancelled; new claims 37-48 added (7 pages)]

31. A method for the detection of a specific nucleic acid sequence in a test sample containing single-stranded DNA which comprises:

(a) contacting the test sample with an oligonucleotide promoter-primer, comprising a promoter for a dedicated RNA polymerase ligated to a primer, under conditions that permit hybridization of the promoter-primer to the nucleic acid sequence to be detected;

(b) contacting the test sample with a DNA polymerase, such that a double-stranded nucleic acid is synthesized, comprising the nucleic acid sequence to be detected and the promoter of the promoter-primer;

(c) contacting the product of step (b) with a dedicated RNA polymerase capable of recognizing the promoter of the oligonucleotide promoter-primer, whereby a multiplicity of RNA transcripts of the nucleic acid sequence to be detected are synthesized by the RNA polymerase under the control of said promoter;

(d) determining the presence of RNA transcripts synthesized in step (c); and

(e) correlating the presence of the RNA transcripts with the presence of the nucleic acid sequence to be detected.

32. A method for the detection of a specific nucleic acid sequence in a test sample containing RNA or single-stranded DNA which comprises:

(a) contacting the test sample with an oligonucleotide promoter-primer, comprising a promoter for a dedicated RNA polymerase ligated to a primer, under conditions that permit hybridization of the promoter-primer to the specific nucleic acid sequence to be detected;

(b) contacting the test sample with reverse transcriptase such that a promoter-primer DNA extension product is synthesized, wherein the template for synthesis of the promoter-primer DNA extension product is the test sample RNA or single-stranded DNA to which the promoter-primer has hybridized in step (a);

(c) treating the product of step (b) under denaturing conditions to separate the promoter-primer DNA extension product from its template;

(d) contacting the single-stranded nucleic acid produced in step (c) with an oligonucleotide secondary primer, wherein said secondary primer is selected to be homologous to all or part of the nucleic acid sequence to be detected and not complementary to the promoter-primer used in step (a), under conditions that permit hybridization of the secondary primer to the promoter-primer DNA extension product;

(e) contacting the product of step (d) with a DNA polymerase or reverse transcriptase, such that a secondary primer DNA extension product is synthesized, wherein the template for synthesis of the secondary promoter DNA extension product is the promoter-primer DNA extension product;

(f) contacting the product of step (e) with a dedicated RNA polymerase capable of recognizing the promoter of the oligonucleotide promoter-primer, whereby a multiplicity of RNA

transcripts of the nucleic acid sequence to be detected are synthesized under the control of said promoter; and

(g) correlating the presence of the RNA transcripts with the presence of the nucleic acid sequence to be detected.

33. A method for the detection of a specific nucleic acid sequence in a test sample containing RNA or single-stranded DNA which comprises:

(a) contacting the test sample with an oligonucleotide secondary primer, under conditions that permit hybridization of the secondary primer to the specific nucleic acid sequence to be detected;

(b) contacting the test sample with reverse transcriptase such that a secondary primer DNA extension product is synthesized, wherein the template for synthesis of the secondary primer DNA extension product is the test sample RNA or single-stranded DNA to which the secondary primer has hybridized in step (a);

(c) treating the product of step (b) under denaturing conditions to separate the secondary primer DNA extension product from its template;

(d) contacting the single-stranded nucleic acid produced in step (c) with an oligonucleotide promoter-primer, comprising a promoter for a dedicated RNA polymerase ligated to a primer, wherein said promoter-primer is selected to be homologous to all or part of the nucleic acid sequence to be detected and not complementary to the secondary primer used in step (a), under conditions that permit hybridization of the promoter-primer to the secondary primer DNA extension product;

(e) contacting the product of step (d) with a DNA polymerase such that a promoter-primer DNA extension product is synthesized, wherein the template for synthesis of the promoter-primer DNA extension product is the secondary primer DNA extension product;

(f) contacting the product of step (e) with a dedicated RNA polymerase capable of recognizing the promoter of the oligonucleotide promoter-primer, whereby a multiplicity of RNA transcripts of the nucleic acid sequence to be detected are synthesized under the control of said promoter; and

(g) correlating the presence of said RNA transcripts with the presence of the nucleic acid sequence to be detected.

34. A method for the detection of a specific nucleic acid sequence in a test sample containing single-stranded DNA which comprises:

(a) contacting the test sample with an oligonucleotide secondary primer, under conditions that permit hybridization of the secondary primer to the specific nucleic acid sequence to be detected;

(b) contacting the test sample with a DNA polymerase such that a secondary primer DNA extension product is synthesized, wherein the template for synthesis of the secondary primer DNA extension product is the test sample RNA or single-stranded DNA to which the secondary primer has hybridized in step (a);

(c) treating the product of step (b) under denaturing conditions to separate the secondary primer DNA extension product from its template;

(d) contacting the single-stranded nucleic acid produced in step (c) with an oligonucleotide promoter-primer, comprising a promoter for a dedicated RNA polymerase ligated to a primer, wherein said promoter-primer is selected to be homologous to all or part of the nucleic acid sequence to be detected and not complementary to the secondary primer used in step (a), under conditions that permit hybridization of the promoter-primer to the secondary primer DNA extension product;

(e) contacting the product of step (d) with a DNA polymerase such that a promoter-primer DNA extension product is synthesized, wherein the template for synthesis of the promoter-primer DNA extension product is the secondary primer DNA extension product;

(f) contacting the product of step (e) with a dedicated RNA polymerase capable of recognizing the promoter of the oligonucleotide promoter-primer, whereby a multiplicity of RNA transcripts of the nucleic acid sequence to be detected are synthesized under the control of said promoter; and

(g) correlating the presence of said RNA transcripts with the presence of the nucleic acid sequence to be detected.

35. A kit for use in the detection of a nucleic acid sequence in a test sample containing an oligonucleotide promoter-primer and an oligonucleotide probe, wherein the promoter-primer is capable of hybridizing to the test sample nucleic acid sequence and the oligonucleotide probe is homologous to all or part of the nucleic acid sequence to be detected.

37. A method for preparing a double-stranded nucleic acid which

includes a promoter operably linked to a sequence to be detected, which method comprises:

(a) providing an oligonucleotide promoter-primer;

(b) contacting said promoter-primer with a nucleic acid comprising the sequence to be detected under conditions that permit hybridization of the promoter-primer to the nucleic acid sequence to be detected;

(c) synthesizing an extension product from the 3' end of the promoter-primer, which extension product is complementary to the nucleic acid sequence to be detected;

(d) contacting the product of step (c) with an agent possessing 3' - 5' exonuclease activity; and

(e) synthesizing an extension product from the 3' end of the sequence to be detected, which extension product is complementary to the promoter of the promoter-primer.

38. The method of claim 37 wherein the promoter-primer of step (a) is the only primer utilized.

39. The method of claim 37 wherein the nucleic acid comprising the sequence to be detected is digested with a restriction enzyme before or during step (b).

40. The method of claims 37 or 38 wherein the synthesis of the extension products of steps (c) and (e) is accomplished using an enzyme selected from the group consisting of E. coli DNA polymerase I, Klenow fragment of E. coli DNA polymerase I, and T4 DNA polymerase.

41. The method of claims 37 or 38 wherein the agent of step (d) is an enzyme selected from the group consisting of E. coli DNA polymerase I, Klenow fragment of E. coli DNA polymerase I, and T4 DNA polymerase.

42. The method of claims 37 or 38 wherein the synthesis of the extension products of step (c) and (e) is accomplished using the same agent as that utilized in step (d).

43. The method of claims 37 or 38 wherein steps (c), (d), and (e) are carried out simultaneously in the same reaction vessel.

44. A method useful for the detection of a specific nucleic acid sequence in a test sample containing nucleic acid, comprising the synthesis of a plurality of RNA transcripts from a double-stranded nucleic acid prepared according to the method of claim 37 or claim 38, wherein each RNA transcript comprises an RNA sequence corresponding to the specific nucleic acid sequence to be detected, and determining the presence of said RNA sequence.

45. The method of claim 44 wherein the plurality of RNA transcripts is synthesized using T7 RNA polymerase.

46. The method of claim 44 wherein the plurality of RNA transcripts is synthesized using SP6 RNA polymerase.

47. The method of claim 44 wherein the presence of said RNA sequence is determined by contacting the RNA transcripts under hybridizing conditions with an oligonucleotide probe selected to hybridize with a predetermined sequence within the RNA transcripts.

48. The method of claim 47 wherein the RNA transcripts are immobilized on a solid support.

STATEMENT UNDER ARTICLE 19

Claims 1-30 and 36 have been cancelled. In their place is added new claims 37-48. The basis for the additional claims is found in the original claims and in the specification, as follows:

Claim	Basis
37	Specification: p.14, line 28-p.16, line 13; Example 1; Fig.1.
38	Specification: p.13, line 26-p.14, line 4; Example 1.
39	Original claim 9.
40	Original claim 11; Specification: p.16, lines 5-9.
41	Specification: p.16, lines 5-9.
42	Specification: p.15, line 34-p.16, line 5.
43	Specification: p.15, line 34-p.16, line 5.

44	Original claim 1; Specification: p.5, lines 24-34; p.16, line 15-p.18, line 27; Fig.1.
45	Original claim 7; p.9, lines 1-5; Example 1.
46	Original claim 8; p. 9, lines 1-5.
47	Original claim 21; Specification: p.17, lines 9-14; p.18, lines 4-18.
48	Original claim 22; Specification: p.18, lines 16-18.

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